EVALUATION OF THE ENTOMOGENOUS FUNGI *CULICINOMYCES CLAVOSPORUS* AND *LAGENIDIUM GIGANTEUM* FOR CONTROL OF THE SALT MARSH MOSQUITO, *AEDES TAENIORHYNCHUS*[1]

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ABSTRACT. In laboratory tests, *Culicinomyces clavosporus* gave similar control (ca 55%) of *Aedes taeniorhynchus* larvae in small containers partially filled with either dredged-spoil (1.8 ppt water salinity) or soil from an upland site (0 ppt water salinity) after 3 days when applied at 1×10^5 conidia/ml. When applied at a rate of 5×10^4 conidia/ml to 5 liters of distilled water in large containers filled with either soil type, *C. clavosporus* resulted in ca 45 and 86% control of *Ae. taeniorhynchus* larvae at days 1 and 5 posttreatment, respectively. Mortality of mosquito larvae in sentinel cages in the large containers of both soil types was 93% at 5 days posttreatment. In field tests, *C. clavosporus* conidia and hyphae applied at a rate of 1×10^{10} conidia/m^2 to the surface of salt marsh pools (water salinities ranged from 10.0 to 13.2 ppt) produced 100% mortality of field-collected, 2nd instar *Ae. taeniorhynchus* larvae in sentinel cages in 3 of 5 treated pools within 24 hr after application, and 98% mortality of larvae in sentinel cages from the remaining pools within 72 hr posttreatment. Dissection and microscopic examination of larvae in laboratory and field tests confirmed infection by *C. clavosporus*, but in the field tests most larvae died before there was extensive mycelial growth of *C. clavosporus* in the hemocoel. *Lagenidium giganteum* did not infect *Ae. taeniorhynchus* larvae in laboratory tests conducted in different-sized containers filled with dredged-spoil (water salinity ranged from 1.6 to 1.9 ppt) and a field test in enclosures in a salt marsh pool (10.0 ppt water salinity).

INTRODUCTION

Brackish water habitats, such as salt marshes (O'Meara 1976) and diked, dredged-material disposal sites (Ezell 1978, Scotton and Axtell 1979, Vorgetts et al. 1980) often produce important pestiferous and disease-vectoring *Aedes* mosquitoes. The development of resistance in mosquito populations to various insecticides, escalating costs, governmental regulations, and public concern for reducing environmental problems caused by insecticide use or habitat alteration have created a need for other methods of mosquito control.

Biological control agents are considered to be important components in an integrated pest management (IPM) approach to mosquito control (Axtell 1979). The entomogenous fungi *Culicinomyces clavosporus* Couch, Romney and Rao and *Lagenidium giganteum* Couch are promising candidates as biological control agents for mosquitoes (Federici 1981). These organisms have not been examined sufficiently under field conditions, however, to justify their use in operational mosquito management programs in coastal areas.

There are two strains of *C. clavosporus:* one from *Anopheles amictus hilli* Woodhill and Lee in Australia (Sweeney et al. 1973) and another from *An. quadrimaculatus* Say in the USA (Couch et al. 1974, Knight 1980). *C. clavosporus* is a virulent pathogen of several genera of Culicidae, Chironomidae, and Ceratopogonidae larvae (Couch et al. 1974, Sweeney 1975b) and can be produced *in vitro*. The Australian isolate of the fungus can tolerate some brackish

[1] Paper No. 8372 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, North Carolina 27650. This research was supported in part by NIH Grant 1RO1 AI 17024.

waters, since in laboratory tests it achieved 100% infection of *An. amictus hilli* in 50% sea water, and 18.5% infection in 75% sea water (Sweeney 1978). In preliminary field tests, *C. clavosporus* completely controlled *Aedes rupestris* Dobrotworsky larvae in several rock pools (Sweeney and Panter 1977). In additional field tests, *C. clavosporus* gave 100% mortality of *Culex quinquefasciatus* Say larvae and 86–100% mortality of *An. annulipes* Walker in artificial ponds, and 90–95% control of *Cx. australicus* Dobrotworsky in a natural pond (Sweeney 1981). Although considerable field and laboratory tests have been conducted with the Australian isolate, similar tests of the USA isolate for mosquito control have not been conducted.

A strain of *Lagenidium* isolated in 1969 from *Culex* larvae (Umphlett and Huang 1970) and later identified as *L. giganteum* (Umphlett 1973) has been shown to be highly lethal to several genera of Culicidae larvae (McCray et al. 1973a, 1973b) and the Clear Lake gnat (Chaoboridae) larvae (Brown and Washino 1979). The fungus can be produced *in vitro*, and overwinters and recycles in some aquatic habitats. Merriam and Axtell (1982) found under laboratory conditions, *L. giganteum* infections by either of 2 different isolates in *Ae. taeniorhynchus* (Wiedemann) were inhibited in waters of very low salinity concentrations (IC_{50} = ca 0.55 parts per thousand (ppt) NaCl). In preliminary field tests, however, McCray et al. (1973b) reported that *L. giganteum* gave variable control of *Cx. tarsalis* Coquillett in rice fields and drainage ditches containing water with salinity concentrations ranging from 0.5 to 12.5 ppt.

The objective of this study was to evaluate USA isolates of *C. clavosporus* and *L. giganteum* for use as microbial control agents for *Ae. taeniorhynchus* larvae in brackish conditions.

MATERIALS AND METHODS

The U.S. strain of *C. clavorsporus* used in this study was isolated in 1977 by A. L. Knight from infected *An. quadrimaculatus* larvae collected near Chapel Hill, NC (Knight 1980). The isolate was routinely maintained on YPSS agar (Difco Laboratories, Detroit, MI).

For laboratory tests, *C. clavosporus* was cultured in corn meal extract (CM) broth. The broth was prepared by adding 20 g of corn meal to 1 liter of distilled water, boiling for 1 hr, and filtering through 3 layers of cheesecloth. Distilled water was added to give a final volume of 1 liter. YPSS agar slices bearing recent fungal growth from the stock cultures were placed into flasks containing 75 ml of sterile CM broth. The flasks were placed on a rotary shaker-table. After growing for 3 weeks at 25–27°C, *C. clavosporus* conidia were harvested from the medium by filtering, centrifugation, and washing 3x with distilled water. Conidia were stored in distilled water for up to 2 weeks at 4°C until use. Concentrations of conidia were determined using a hemocytometer. In order to increase *C. clavosporus* production for field tests, the fungus was grown in culture flasks containing 500 ml CM broth. Since conidia tended to adhere to the cheesecloth during the filtration stage of harvesting, this step was omitted. Instead the fungus was homogenized briefly in order to break up the mycelial growth, and the conidia and hyphal segments were harvested by centrifugation and washing 3x with distilled water, and stored as before.

The isolate of *L. giganteum* used in this study was isolated by A. L. Knight from infected mosquito larvae in Chapel Hill, NC in 1979, and was routinely maintained on Peptone-Yeast Extract-Glucose (PYG) agar (0.125% peptone, 0.125% yeast extract, 0.3% glucose and 2.0% agar).

For laboratory and field tests, *L. giganteum* was cultured following methods similar to those used by Merriam and Axtell (1982). PYG agar slices bearing recent mycelial growth from the stock cultures were placed into flasks containing 75 ml of sterile HWYG broth (hemp seed

extract at 0.25 mg soluble protein/ml broth, 0.32% wheat germ, 0.14% yeast extract, and 0.12% glucose). The flasks were placed on a rotary shaker-table. After growing for 5 days at 25–27°C, the fungus was washed 2x with sterile, distilled water and homogenized briefly to break the mycelia into segments of a few cells each. Several, 100-mm-diam petri dishes, each containing ca 20 ml of whole hemp seed extract (WHS) agar (1.0 mg soluble protein/ml), were inoculated with ca 1.5 ml of washed fungus. The fungus was grown for 6 to 8 days prior to testing.

Laboratory tests to determine the efficacy of *C. clavosporus* and *L. giganteum* for control of *Ae. taeniorhynchus* larvae in simulated coastal habitats were conducted in both small (17 cm diam × 13 cm depth) and large (32 cm diam × 21 cm depth) containers. The containers were partially filled with soil from either a diked, dredged-spoil disposal site (No. 12) located along the Atlantic Intracoastal Waterway in Brunswick Co., NC, or an upland site located in Wake Co., NC. Neither site had received a pesticide treatment prior to the collection of soil samples. The soil from each site was sterilized and placed into small and large containers to depths of 5 and 10 cm, respectively.

Small containers were flooded with distilled water to give a standing water volume of 1 liter (4.4 cm depth). Distilled water was added daily to each container in order to compensate for evaporative losses. There were 4 "upland" and 4 "dredged-spoil" containers for each fungus treatment, and 4 untreated containers (2 of each soil type) as controls. Twenty-four hours after flooding the salinity of the water in each container was measured. Eighty 24-hr-old *Ae. taeniorhynchus* larvae from a laboratory colony were added to the water in each container. The larvae were fed powdered rabbit chow and brewer's yeast. The agar in 2 petri dishes bearing recent fungal growth was cut into several pieces and added to the water in each of the *L. giganteum*-treated containers. This amount of *L. giganteum* represented a potential dose rate of 2450 zoospores/ml water in each container. Larvae were removed from the containers after 72 hr and examined microscopically for signs of infection by *L. giganteum*. The criterion for *L. giganteum* infection was the visual presence of hyphae or sporangia in the head capsules and thoraces of the larvae. In each of the *C. clavosporus*-treated containers, conidia were pipetted to the surface of the water to give a final concentration of 1×10^5 conidia/ml. Care was taken in order to apply the conidia as uniformly as possible. Percent mortality was determined after 3 days by counting the number of live larvae remaining in each container. Living and dead larvae were removed, dissected and examined microscopically for evidence of *C. clavosporus* infection. The criteria for infection were the visual presence of hyphal penetration through the foregut or hindgut, and growth in the hemocoel. Mortalities in the treated containers were corrected for mortalities in the untreated controls by Abbott's formula.

Additional tests were conducted in large containers 24 hr after they were flooded with distilled water to give a standing water volume of 5 liters (6.2 cm depth). There were 4 containers for each soil type and treatment, and 4 untreated containers (2 of each soil type) as controls. In the *L. giganteum* treatments and controls, sentinel cages containing 50, 48-hr-old *Ae. taeniorhynchus* larvae were placed into the water in each container. The sentinel cages were constructed from clear, plastic jars (9 cm diam × 14 cm depth) which were fitted with an 8-cm-wide brass screen (23 holes/cm) on its side. The bottoms of the sentinel cages were not removed so that the cages would retain ca 125 ml of water when removed from the test containers. The agar in 3 petri dishes bearing recent mycelial growth of *L. giganteum* was cut into several pieces and added to the water in each of the *L. giganteum*-treated containers, representing a potential dose rate of 735 zoospores/ml water. Larvae were re-

moved from the sentinel cages 72 hr after treatment and examined microscopically for signs of *L. giganteum* infection. In the *C. clavosporus* treatments and controls, ca 500, 48-hr-old *Ae. taeniorhynchus* larvae were added to the water in each container, along with sentinel cages containing 50 larvae. *Culicinomyces clavosporus* conidia were pipetted to the surface of the water in the treated containers to give a final concentration of 5×10^4 conidia/ml. Mean numbers of mosquito larvae present in the containers were based on 10 dips with a standard enameled dipper before treatment and at 1, 3 and 5 days posttreatment. Percent mortality of larvae in sentinel cages was determined after 5 days of counting the number of live larvae remaining in each sentinel cage. Living and dead larvae were examined for evidence of *C. clavosporus* infection. Mortalities in treated containers were corrected for mortalities in the controls by Abbott's formula.

Field tests of *L. giganteum* and *C. clavosporus* were conducted during October, 1981, in salt marsh pools in Vandemere and Hobucken, Pamlico Co., NC. At each site, the pools were isolated from one another and ranged in size from 0.2 to 2.1 m^2, and were 2.5 to 10.2 cm deep on the 1st day of the tests. Second instar *Ae. taeniorhynchus* larvae were present in each pool. Water salinity and temperature were recorded using field instruments. Water temperatures ranged from 11.1 to 27.8°C, and salinities ranged from 10.0 to 13.7 ppt. *Culicinomyces clavosporus* and *L. giganteum* were transported to the field sites in a cooler which was partially filled with ice. Fifty mosquito larvae were collected from each pool and placed into sentinel cages which were anchored to the marsh substrate in each pool with stakes. *Culicinomyces clavosporus* conidia and hyphae were applied evenly to the surface of the water in each treated pool using a hand-pumped, compressed air sprayer on the basis of water surface area (1×10^{10} conidia/m^2). A sample of the inoculum from the sprayer was returned to the laboratory to test for virulence using field-collected larvae. Since many pools had dried within 24 hr after treatment, the water and mosquito larvae remaining in the bottom of the sentinel cages in the treated and control pools were collected and returned to the laboratory and placed in glass beakers. Larvae were fed ground rabbit chow and brewer's yeast. Cumulative mortalities were determined at 1, 2 and 3 days posttreatment by counting the numbers of live larvae in each beaker. Dead larvae were dissected and examined daily (live larvae on day 3) for evidence of infection by *C. clavosporus*.

Lagenidium giganteum was tested in one of the larger, deeper pools at the Vandemere site. The salinity in this pool was 10.0 ppt at the beginning of the test. Tests were conducted in plastic enclosures, which were the large containers used in the laboratory tests with their bottoms removed. Five enclosures were pushed into the marsh substrate in the pool and anchored with stakes. Fifty 2nd instar mosquito larvae were collected from the pool and placed into a sentinel cage in each enclosure. The agar bearing *L. giganteum* in 4 petri dishes was cut into several sections and placed into the water in each of 3 treated enclosures. The other 2 enclosures were untreated as controls. Several of the petri dishes containing *L. giganteum* not used in the field test were returned to the laboratory, where the fungus was tested for virulence in distilled water against field-collected mosquito larvae. All of the larvae in the sentinel cages, and samples of larvae from the enclosures were removed from the treated and control enclosures 48 hr after treatment. These larvae were returned to the laboratory where they were examined microscopically for signs of *L. giganteum* infection.

RESULTS

The mortalities of free-swimming and sentinel *Ae. taeniorhynchus* larvae after introduction of *C. clavosporus* conidia into different volumes of water in containers partially filled with soil from either a

diked, dredged-spoil site or an upland site are shown in Table 1. Water salinity did not adversely affect the ability of *C. clavosporus* to infect mosquito larvae in the 2 different volumes of distilled water, since the percent mortality of mosquito larvae in the containers filled with each soil type was very similar. Upon dissection of some of the mosquito larvae remaining alive in the 1 liter containers 72 hr after treatment with *C. clavosporus* conidia, 34% of the larvae in the "dredged-spoil" containers and 26.7% of the larvae in the "upland" containers were found to be infected with the fungus. In the infected larvae, *C. clavosporus* hyphae had penetrated through the foregut or hindgut, and the mycelium was growing in the hemocoel. Although several of the dead larvae examined also had mycelial growth in their body cavities, most of the dead larvae apparently died in considerably less than 72 hr since the cadavers were being consumed by other microorganisms, and thus infection by *C. clavosporus* in these larvae could not be positively confirmed. The percent mortality of mosquito larvae in sentinel cages in 5 liter containers of both soil types at 5 days posttreatment was ca 93%. While most of the cadavers in the sentinel cages were overwhelmed by other microorganisms, several larvae were filled with *C. clavosporus*, which was sporulating. Of the few larvae remaining alive in the sentinel cages in the treatments, none showed signs of infection by *C. clavosporus* upon dissection.

The results of tests using *C. clavosporus* for control of free-swimming *Ae. taeniorhynchus* larvae in containers partially filled with soil from either a diked, dredged-spoil site or an upland site and flooded with 5 liters distilled water are shown in Table 2. There was little difference between the percent control of mosquito larvae by *C. clavosporus* in water with 1.8 ppt salinity, and that achieved by the fungus in water with 0 ppt salinity. The average no. of larvae/dip in treated containers of both soil types declined ca 45% within 1 day following application of *C. clavosporus* conidia, and reached ca 86% by day 5 posttreatment. Most of the larvae which appeared dead on day 3 posttreatment in the treated containers were infected by *C. clavosporus*. These larvae each had hyphal growth through the foregut or hindgut, and into the hemocoel. On day 5 posttreatment, the dead larvae were either consumed by other microorganisms, or had sporulating *C. clavosporus* on the surface of their exterior cuticles.

The cumulative percent mortality of caged, field-collected *Ae. taeniorhynchus*

Table 1. Mortality of free-swimming and sentinel *Aedes taeniorhynchus* larvae after introduction of *Culicinomyces clavosporus* conidia[a] into different volumes of distilled water in containers partially filled with soil from either a diked, dredged-spoil site (Brunswick Co., NC) or an upland site (Wake Co., NC).

Volume of water (liter)	Soil type	Mean salinity (ppt)	Mean mortality[b] % (+ S.E.)	Live larvae dissected infected/total
1	Dredged-spoil	1.8	57.7 (5.9)	12/35
	Upland	0	53.5 (11.9)	8/30
5	Dredged-spoil	1.8	93.0 (3.5)	0/13
	Upland	0	92.6 (2.7)	0/10

[a] *C. clavosporus* conidia were applied at 1×10^5 conidia/ml to the water in small containers with 1 liter of water, while large containers with 5 liters of water received 5×10^4 conidia/ml.

[b] Percent mortality was determined at 3 days posttreatment in the 1 liter containers and at 5 days posttreatment in the 5 liter containers using Abbott's formula. There were 80 free-swimming 24-hr-old mosquito larvae in each container filled with 1 liter of water, while there were 50, 48-hr-old sentinel mosquito larvae in each of the containers filled with 5 liters of water. There were 4 replicates per soil type at each volume of water.

Table 2. Comparison of effectiveness of *Culicinomyces clavosporus* conidia for control of free-swimming *Aedes taeniorhynchus* larvae in containers partially filled with soil from either a diked, dredged-spoil site (Brunswick Co., NC) or an upland site (Wake Co., NC), and flooded with 5 liters of distilled water.

Soil type	Dose (conidia/ml)	Salinity (ppt)	Ave. no. larvae/dip (% control)[a]			
			Pre-treatment	Days posttreatment		
				1	3	5
Dredged-spoil	5×10^4	1.8	42.7	24.6(48.5)	15.6(56.9)	6.0(82.4)
Dredged-spoil	untreated	1.9	40.5	45.3	34.8	32.4
Upland	5×10^4	0	57.5	33.5(44.8)	18.0(68.6)	5.1(90.3)
Upland	untreated	0	30.8	32.5	30.7	28.3

[a] Average no. larvae per dip based on 10 dips with a standard enameled dipper in each container. Percent control was calculated using Abbott's formula.

larvae after 24 hr exposure to *C. clavosporus* in salt marsh pools is shown in Table 3. Within 24 hr after *C. clavosporus* application, 100% of the larvae in the sentinel cages in most treated pools were dead and, upon dissection, were found to be infected by the fungus. In each of these larvae, there were several *C. clavosporus* conidia which had germinated and penetrated the foregut. The larvae apparently died, however, before the hemocoel was colonized by the mycelium. The alimentary tract of each larva was also completely filled with *C. clavosporus* conidia and hyphae, although the conidia had not germinated in the midgut. In addition, many conidia and hyphae were adhered to the mouthparts of the larvae, in particular the oral vibrissae. Those larvae which were held in the laboratory and died on days 2 and 3 posttreatment also showed signs of *C. clavosporus* infection, and had mycelial growth in their body cavities. In both cases, the dead larvae were also being consumed by other microorganisms. The larvae which remained alive on day 3 posttreatment showed no signs of infection by *C. clavosporus* upon dissection.

Pretreatment dip counts taken in pool

Table 3. Cumulative percent mortality of caged, field-collected 2nd instar *Aedes taeniorhynchus* larvae after exposure to *Culicinomyces clavosporus* for 24 hr in salt marsh pools in Pamlico Co., NC (Oct. 21, 1981), and subsequently reared in the laboratory.

Test location	Replicate (pool)[a]	Treatment (conidia/m^2)	Salinity (ppt)	Cumulative % mortality[b]		
				days posttreatment		
				1	2	3
Vandemere, NC	1	1×10^{10}	12.0	100	—	—
	2	1×10^{10}	11.3	72	80	98
	3	1×10^{10}	13.2	100	—	—
	4	untreated	13.0	0	0	2
	5	untreated	11.3	2	2	4
Hobucken, NC	6	1×10^{10}	12.0	94	98	98
	7	1×10^{10}	10.0	100	—	—
	8	untreated	13.7	0	0	2
	9	untreated	13.0	0	0	8

[a] Pools ranged in size from 0.2 to 2.1m^2, and 2.5 to 10.2 cm deep on the day of *C. clavosporus* application. Pools were isolated from one another.
[b] Mortality based on 50 sentinel mosquito larvae per pool.

no. 6 at Hobucken, NC averaged 94 larvae/dip, while 24 hr after *C. clavosporus* application, dip counts in this pool averaged 11 larvae/dip. This equalled an 85.6% reduction in mosquito abundance when corrected for an 18.9% reduction in mosquito abundance in control pools using Abbott's formula. No dip counts were taken on day 2 posttreatment since the pool was dry. Pool No. 7 was dry within 24 hr posttreatment.

The results of tests using *L. giganteum* for control of laboratory-reared *Ae. taeniorhynchus* larvae in different volumes of distilled water in containers partially filled with either dredged-spoil or soil from an upland site are shown in Table 4. In the small containers flooded with 1 liter of distilled water, *L. giganteum* infected most of the mosquito larvae in the "upland" containers, but was unable to infect mosquito larvae in "dredged-spoil" containers with a mean water salinity of 1.9 ppt. Similar results were obtained in the larger containers of both soil types flooded with 5 liters distilled water. The mean percent infection in mosquito larvae by *L. giganteum* was less in the 5 liter "upland" containers than was the case in the respective 1 liter containers, and this may be due to lower dose rates in the 5 liter containers, or possible protection offered by the sentinel cages to mosquito larvae as the cages restricted the movement of larvae in the large containers. *L. giganteum* infections in mosquito larvae in the "upland" containers of both sizes were at Stage II infection (Domnas et al. 1974).

Table 4. Comparison of infection of laboratory-reared *Aedes taeniorhynchus* larvae following introduction of *Lagenidium giganteum*[a] into different volumes of distilled water in containers filled with either soil from a diked, dredged-spoil site (Brunswick Co., NC) or an upland site (Wake Co., NC).

Volume of water (liter)	Soil type	Mean salinity (ppt)	Mean infection[b] % ± (S.E.)
1	Dredged-spoil	1.9	0
	Upland	0	96.5 (1.7)
5	Dredged-spoil	1.6	0
	Upland	0	41.0 (7.9)

[a] Containers filled with 1 liter distilled water were inoculated with *L. giganteum* mycelia growing on the agar in 2, 100-mm-diameter petri dishes, which represents a potential dose rate of 2450 zoospores/ml. Containers filled with 5 liters distilled water were inoculated with *L. giganteum* mycelia growing on the agar in 3 petri dishes, which represents a potential dose rate of 735 zoospores/ml.

[b] Means based on 80 free-swimming mosquito larvae per 1 liter container and 50 sentinel mosquito larvae per 5 liter container exposed 72 hr. There were 4 replicates per soil type at each water volume.

In a field test conducted in a salt marsh pool with a water salinity of 10.0 ppt at Vandemere, NC, *L. giganteum* failed to infect larvae in both the sentinel cages and enclosures. A portion of the *L. giganteum* inoculum not used in the field test and returned to the laboratory for testing in distilled water resulted in 97% infection in field-collected *Ae. taeniorhynchus* larvae.

DISCUSSION AND CONCLUSIONS

The results of this study show that *C. clavosporus* is a promising microbial control agent for mosquito larvae under some brackish water conditions. The USA isolate of *C. clavosporus* appears to have a similar salinity tolerance to that of the Australian isolate, since in our study the USA isolate infected mosquito larvae under laboratory conditions in water with salinity concentrations of ca 1.8 ppt, and under field conditions in waters with salinites ranging from 10.0 to 13.2 ppt. Sweeney (1978) studied the effects of salinity on the Australian isolate of *Culicinomyces* and found that the fungus killed 100% of *An. amictus hilli* in 50% sea water under laboratory conditions.

The high mortality of *Ae. taeniorhynchus* larvae after 5 days in containers treated with *C. clavosporus* at 5×10^4 conidia/ml is similar to that reported by Sweeney and Panter (1977). They found that 100% and 89% of *Ae. rupestris* larvae were killed in 5

days when exposed to *Culicinomyces* conidia at 1×10^5 conidia/ml in containers filled with 4 liters of water in the laboratory and field, respectively. In tests conducted in natural pools, Sweeney and Panter (1977) reported that all of the *Ae. rupestris* larvae died within 5 days following application of *Culicinomyces* conidia at 1×10^5 and 1×10^6 conidia/ml. Thus, the high mortality we observed in sentinel larvae within 24 hr after exposure to *C. clavosporus* conidia at 1×10^{10} conidia/m^2 (equivalent to ca 1×10^5 to 5×10^5 conidia/ml in each pool) in our field tests was unexpected. Subsequent laboratory tests to ascertain whether the hyphae, which were applied with the conidia, were toxic to *Ae. taeniorhynchus,* or if the field inoculum was contaminated by pesticides were negative. Sweeney (1975a) studied the mode of infection of *Culicinomyces* in *Culex quinquefasciatus* Say (= *fatigans*) larvae and found that most larvae died when the hemocoel was completely filled with the fungus. Sweeney (1975a) indicated, however, that some mosquitoes died soon after the fungus penetrated the foregut or hindgut, or during early hyphal growth in the body cavity. This was apparently the case with the *Ae. taeniorhynchus* larvae in our field tests, since the larvae showed signs of fungal penetration, but had died before extensive hyphal growth in the body cavity had occurred. While the exact cause of the rapid mortality in these larvae is unknown, it may be due to the possible production of toxic substances, as mentioned by Couch et al. (1974). Toxic substances may be associated with penetrant hyphae, and when large numbers of conidia give rise to a massive invasion of the foregut or hindgut, a lethal titer of toxins in the hemocoel may cause rapid larval death (A. W. Sweeney, personal communication).

Our laboratory and field tests of the North Carolina isolate of *L. giganteum* show that it is not an effective microbial control agent for mosquitoes in brackish water habitats. Merriam and Axtell (1982) examined the salinity tolerance of this isolate and a Louisiana isolate of *L. giganteum* under laboratory conditions. They found that there was complete inhibition of zoosporogenesis and mosquito infection in both isolates in water containing 1.5 ppt NaCl, and the IC_{50} values for inhibition of infection in mosquito larvae by the LA and NC isolates were 0.52 and 0.55 ppt NaCl, respectively. In field tests conducted in rice-field seepage ditches using a different isolate of *L. giganteum* for control of *Cx. tarsalis,* however, McCray et al. (1973b) reported infectivities of 100% in water with 0.5 ppt NaCl, and 8.8% in water with 7.2 ppt NaCl. In light of their results and ours, further field tests using various isolates (including possibly laboratory selected ones) should be conducted in brackish water habitats before totally ruling out the use of *L. giganteum* under saline conditions.

ACKNOWLEDGMENTS

This study was made possible by the cooperation of Mr. Al Propst, Pamlico County, NC Mosquito Control Program. Assistance of Dr. Stefan Jaronski, Department of Entomology, N.C.S.U., Raleigh, NC in culturing *L. giganteum* and *C. clavosporus* is gratefully acknowledged.

Literature Cited

Axtell, R. C. 1979. Principles of integrated pest management (IPM) in relation to mosquito control. Mosq. News 39:709–18.

Brown, J. K. and R. K. Washino. 1979. Evaluating the fungus *Lagenidium giganteum* for the biological control of the Clear Lake gnat, *Chaoborus astictopus,* in an agricultural pond in Lake County, California. Proc. Calif. Mosq. Control Assoc. 47:37 (abstract).

Couch, J. N., S. V. Romney and B. Rao. 1974. A new fungus which attacks mosquitoes and related Diptera. Mycologia 66:374–9.

Domnas, A., P. E. Giebel and T. M. McInnis, Jr. 1974. Biochemistry of mosquito infection: preliminary studies of biochemical change in *Culex pipiens quinquefasciatus* following infection with *Lagenidium giganteum.* J. Invertebr. Pathol. 24:293–304.

Ezell, B. W., Jr. (ed.) 1978. An investigation of

physical, chemical and/or biological control of mosquitoes in dredged material disposal areas. U.S. Army Engineers Waterways Expt. Sta. (Vicksbuirg, Miss.) Tech. Rpt. D–78–48. 266 pp.

Federici, B. A. 1981. Mosquito control by the fungi *Culicinomyces, Lagenidium* and *Coelomomyces*. In: Microbial control of pests and plant diseases 1970–1980. (H. D. Burges, ed.) Academic Press, New York. p. 555–72.

Knight, A. L. 1980. Host range and temperature requirements of *Culicinomyces clavosporus*. J. Invertebr. Pathol. 36:423–25.

McCray, E. M., Jr., C. J. Umphlett and R. W. Fay. 1973a. Laboratory studies on a new fungal pathogen of mosquitoes. Mosq. News 33:54–60.

McCray, E. M., Jr., D. J. Womeldorf, R. C. Husbands and D. A. Eliason. 1973b. Laboratory observations and field tests with *Lagenidium* against California mosquitoes. Proc. Calif. Mosq. Control Assoc. 41:123–8.

Merriam, T. L. and R. C. Axtell. 1982. Salinity tolerance of two isolates of *Lagenidium giganteum* (comycetes: Lagenidiales), a fungal pathogen of mosquito larvae. J. Med. Entomol. 19:388–93.

O'Meara, G. F. 1976. Saltmarsh mosquitoes (Diptera: Culicidae). p. 303–33. In: Marine insects. (L. Cheng. ed.) North-Holland Publishing Co., Amsterdam. 581 pp.

Scotton, G. L. and R. C. Axtell. 1979. *Aedes taeniorhynchus* and *Ae. sollicitans* (Diptera: Culicidae) oviposition on coastal dredge spoil. Mosq. News 39:97–110.

Sweeney, A. W. 1975a. The mode of infection of the insect pathogenic fungus *Culicinomyces* in larvae of the mosquito *Culex quinquefasciatus* (= *fatigans*). Aust. J. Zool. 23:49–57.

Sweeney, A. W. 1975b. The insect pathogenic fungus *Culicinomyces* in mosquitoes and other hosts. Aust. J. Zool. 23:59–64.

Sweeney, A. W. 1978. The effects of salinity on the mosquito pathogenic fungus *Culicinomyces*. Aust. J. Zool. 26:55–9.

Sweeney, A. W. 1981. Preliminary field tests of the fungus *Culicinomyces* against mosquito larvae in Australia. Mosq. News 41:470–6.

Sweeney, A. W. and C. Panter. 1977. The pathogenicity of the fungus *Culicinomyces* to mosquito larvae in a natural field habitat. J. Med. Entomol. 14:495–6.

Sweeney, A. W., D. J. Lee, C. Panter and L. W. Burgess. 1973. A fungal pathogen for mosquito larvae with potential as a microbial insecticide. Search (Sidney) 4:344–5.

Umphlett, C. J. 1973. A note to identify a certain isolate of *Lagenidium* which kills mosquito larvae. Mycologia 65:970–71.

Umphlett, C. J. and C. S. Huang. 1970. *Lagenidium culicidum* as an agent of biological control of mosquitoes. Bull. Assoc. S. East. Biol. 17:68.

Vorgetts, J., Jr., W. B. Ezell, Jr. and J. D. Campbell. 1980. Species composition of mosquitoes produced in dredged material, wildlife management, and natural saltmarsh habitats of the South Carolina coast. Mosq. News 40:501–6.

ERRATUM

The following table should be substituted for Table 2 in the article by R. J. Sebastian and R. A. Brust, 1981 Mosquito News, Vol. 41(3):510.

Table 2. Efficacy of *Bacillus thuringiensis israelensis* (Biochem) on 2nd and 4th instar larvae of *Culex restuans* and *Aedes vexans*. Mean water temperature 19.0°C.

Species	Water pH	Percent mortality 0.1 mg/liter 12 h	24 h	48 h	0.4 mg/liter 12 h	24 h	48 h	0.8 mg/liter 12 h	24 h	48 h	Control 48 h
Culex restuans											
2nd instar	7.1	23	38	38	50	75	80	55	68	78	8
4th instar	7.1	25	38	63	95	98	100	100	100	100	15
Aedes vexans											
2nd instar	6.9	33	53	75	90	100	100	100	100	100	18
4th instar	6.9	8	25	43	60	83	93	88	95	100	8